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CHANGES IN THE MICROBIOLOGICAL AND SHELF-LIFE CHARACTERISTICS OF BEEF TONGUES AND LIVERS FOLLOWING TRANSCONTINENTAL AND TRANSOCEANIC SHIPMENT

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SUMMARY

The effects of transcontinental (interstate) transport and transoceanic shipment were determined on microbiological and shelf-life characteristics of beef tongues and livers. These variety meats were evaluated for both microbiological and shelf-life characteristics following slaughter in Guymon, Oklahoma, USA. The samples were then frozen and sent by refrigerated truck (4·5°C) to storage facilities in Jacksonville, Florida, USA. Further microbiological and shelf-life evaluations took place prior to overseas shipment at the University of Florida and following overseas shipment at The Institute CIVO-Technology, TNO, Zeist, The Netherlands. Aerobic plate counts (APCs) at 35°C for beef tongues showed a significant ($P < 0·05$) *decrease following overseas shipment. Similar results were noted for beef livers. For both organs, the surface thawing in Florida, required for sampling, did not appear to affect the final bacterial counts. The 20°C APCs for beef tongues revealed a*

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significant ($P < 0{\cdot}05$) *decrease following interstate transport but not transoceanic shipment. The 20°C APCs for beef livers did not differ significantly during the entire transportation period. The colour differences noted during transport of the product were probably the result of freezing and not of the actual shipping and storage conditions.*

INTRODUCTION

The United States is a major supplier of variety meats (edible offal) to the European Market. In 1979, the US supplied 30·9 % of the total beef and pork variety meats to the European Economic Community (Miller & Bongers, 1981). This market is a valuable outlet for US variety meats for which the demand is very limited in the United States. However, several problems have been occasionally noted with the exporting of US variety meats to Europe. These include: (1) failure to adhere to product quality standards and specifications; (2) deterioration of product and packaging during transit; (3) inappropriate processing, chilling and freezing procedures and (4) uncontrolled systems of product assembly for export by brokers.

There appears to be no published data on the microbial and shelf-life changes occurring with variety meats following transcontinental (interstate) and transoceanic shipment. Marriott *et al.* (1977*a*) noted that during long-distance transoceanic shipment of fresh beef (21 days' duration) bacterial numbers were significantly ($P < 0{\cdot}05$) lower on samples placed in a refrigerated van with a modified atmosphere (60 % CO_2, 25 % O_2, 15 % N_2) than on those shipped under normal atmospheric conditions. Marriott *et al.* (1977*b*) also found that the modified atmosphere allowed for lower bacterial numbers than the normal atmosphere during a short-term (7–9 days) transoceanic shipment. They found final bacterial counts to be unaltered either by spray coating with calcium alginate, wrapping in PVC film, vacuum packaging, or leaving the product unwrapped in polyethylene-lined boxes.

Only limited information on microbial growth patterns of variety meats has appeared in the literature. Aerobic counts for freshly slaughtered variety meats have been 10^3–10^4 per either gram or square centimetre. Gardner (1971) found the microbial contamination on pork livers to be predominantly on the surface of the livers and that freezing did not change the microbial spoilage patterns. Shelef (1975) noted that storage of fresh beef liver for 7–10 days at 5 °C produced a sour odour which was attributed to a predominance of lactic acid bacteria. Beef variety meats obtained under normal commercial conditions in abattoirs and then subjected to 2–3 weeks storage at 1 ± 1 °C in vacuum packages have been shown to have only 3–4 days of shelf-life at 4 °C once exposed to air (Patterson & Gibbs, 1979). Vacuum packaging has been shown to decrease *Enterobacteriaceae*, but increase *Lactobacillacea* counts on pork liver (de Kruijf, 1979).

The purpose of this study was to analyse the changes in the microbial and shelf-life characteristics of frozen beef tongues and livers as a result of domestic transport at above freezing temperatures from slaughter plant to the shipping port, followed by transoceanic shipment to an overseas port.

MATERIALS AND METHODS

Sample acquisition

Beef livers and tongues were selected and evaluated for microbiological and shelf-life characteristics at a large meat packing plant in Guymon, Oklahoma, USA. Tongues were chilled 24 ± 3 h at 4–5 °C before evaluation. They were trimmed of excess fat and placed in two layers in boxes; approximately sixteen per box, depending on the size of the tongues. Beef tongues were 24 ± 3 h post-evisceration at time of sampling. Procedures for the handling of beef livers varied quite significantly from that of beef tongues. Handling procedures for beef livers prior to evaluation were as follows: (1) Livers were excised from animals and placed on stainless steel offal racks, (2) they were then washed and placed in individual plastic bags, replaced on the rack and (3) rolled into a blast freezer (−35 °C to −40 °C) for a period of 3–5 h prior to evaluation. Livers were then packaged two per box. At the time of sampling, beef livers were 3–5 h post-evisceration.

Sampling procedure

Each variety meat was sampled for surface bacteria. A sterile dacron-tipped swab, moistened in 0·1 % peptone (Difco) diluent was used to assess bacteria on 12·3 cm^2 of the surface according to the procedures of Lazarus *et al.* (1977). Three separate 12·3 cm^2 areas were swabbed with individual sterile swabs. The three swabs were then pooled into 10 ml of 0·1 % peptone diluent and appropriate serial dilutions were made. Aerobic plate counts at 20 °C were the only microbial analyses performed in Guymon, due to limited laboratory facilities (Table I).

TABLE I
MICROBIOLOGICAL DETERMINATIONS

Determination	*Medium*	*Plating technique*	*Incubation*	*Confirmation test*
Aerobic plate count (35 °C)	Plate count agar	Pour plate	35 °C/48 h	–
Aerobic plate count (20 °C)	Plate count agar	Pour plate	20 °C/5 days	–
Aerobic plate count (7 °C)	Plate count agar	Pour plate	7 °C/10 days	–
Coliform count	Violet red bile agar	Pour plate with overlay	35 °C/24 h	Growth + gas evolution in brilliant green bile broth within 48 h at 35 °C
Total *Enterobacteriaceae* count	Violet red bile agar with 1 % glucose	Pour plate with overlay	35 °C/24 h	–

Panel evaluations

A two-member panel, trained in the evaluation of shelf-life characteristics, evaluated the variety meat samples initially at the plant in Guymon. Beef tongues were evaluated for off-odour, and colour of exposed muscle, while beef livers were scored for surface colour, colour uniformity and off-odour. All organs were weighed following sampling. The scoring systems are given in Table 2.

TABLE 2
SCORING SYSTEMS FOR SHELF-LIFE STUDY

Off-Odour	*Uniformity of colour*	*Colour*[a]
4 = No off-odour	8 = Very uniform	1 = light brownish orange, colour chip 5YR 5/6
		2 = strong yellowish brown, colour chip 10YR 5/6
3 = Slight off-odour	7 = Uniform	3 = light yellowish brown, colour chip 7·5YR 5/6
		4 = strong brown, colour chip 7·5YR 4/6
2 = Moderate off-odour	6 = Moderately uniform	5 = light brown, colour chip 5YR 5/4
		6 = light reddish brown, colour chip 10R 5/4
1 = Extreme off-odour	5 = Slightly uniform	7 = moderate reddish brown, colour chip 10R 4/4
		8 = greyish red, colour chip 7·5R 4/4
	4 = Slightly uneven	9 = moderate reddish brown, colour chip 7·5R 3/6
		10 = moderate reddish brown, colour chip 10R 3/6
	3 = Moderately uneven	11 = moderate reddish brown, colour chip 7·5R 3/4
		12 = dark red colour chip 5R 3/4
	2 = Uneven	13 = blackish purple, colour chip 5RP 2/1
		14 = moderate reddish brown, colour chip 2·5YR 3/4
	1 = Very uneven	15 = moderate olive, colour chip 5Y 4/4
		16 = vivid red, colour chip 7·5R 3/12
		17 = dark purplish red, colour chip 10RP 2/6
		18 = moderate red, colour chip 5R 4/10
		19 = dark reddish grey, colour chip 10R 4/1
		20 = moderate reddish brown, colour chip 10R 4/6
		21 = very dark red, colour chip 5R 2/6

[a] Numerical scores on the left refer to numbers assigned by the panel; the remaining information refers to the Munsell (Munsell Company, Baltimore, MD) colour chips used as standards for the numerical scale.

Transcontinental shipment

The beef tongues and livers were palletised and placed in a blast freezer (-30 to -40 °C) for a period of 7 days. The samples were then transported 2264 km to Jacksonville, Florida, USA, by refrigerated tractor-trailer. The temperature inside the trailer was maintained at approximately $4{\cdot}5\,°C \pm 2\,°C$. Total transportation time was 72 h.

Sampling: Sixteen of forty-two livers and fourteen of sixty tongues were transported 160 km from Jacksonville, to the University of Florida (Gainesville, Florida), where these organs were subjected to microbiological and shelf-life evaluation. Following evaluation, these organs were reinserted into the test shipment.

Microbiological and shelf-life procedures in Florida: Microbiological analyses included the following: aerobic plate counts at 7°, 20° and 35 °C, coliform counts, and total *Enterobacteriaceae* counts. A summary of these procedures is given in Table 1.

Panel evaluations: Organoleptic evaluations were performed on the product immediately following microbial sampling by the same two-member panel who evaluated the product in Guymon. These procedures have previously been described.

Transoceanic shipment

The boxed variety meats were held in frozen storage for 165 days at Jacksonville (−18 °C) and Gainesville (−13 °C) prior to shipping to Tampa, Florida, by refrigerated truck (held at a frozen temperature). The product was stored 19 days in a cold storage warehouse prior to being shipped to Rotterdam, The Netherlands. The boxes of variety meats were shipped break bulk (non-containerised, boxes stacked) on a single pallet. Transoceanic transport to Rotterdam took 11 days, after which the boxes were transferred 120 km to the Institute CIVO-Technology TNO in Zeist, The Netherlands. During transit, the pallet was packed in dry ice aboard a non-refrigerated truck. Samples were reassessed for microbiological and shelf-life properties at Zeist.

Handling, microbiological and panel evaluation procedures: Samples were held 6 days at −20 °C at the laboratory in Zeist, before being thawed. Beef tongues were thawed 8 h at 10 °C followed by 24 h at 0–1 °C while beef livers were thawed 24 h at 0–1 °C.

Microbiological analyses included the following: aerobic plate counts at 7°, 20° and 35 °C; coliform counts and total *Enterobacteriaceae* counts (Table 1).

Panel evaluations: Beef livers were subjectively evaluated for odour, colour and uniformity of colour while beef tongues were evaluated for odour and colour of exposed muscle using procedures previously described. Evaluations were performed by a different two-member panel from the one which evaluated product in the United States.

Statistical analysis: The analysis of variance (ANOVA) procedure (Snedecor & Cochran, 1972) was used to test the effects of interstate transport and transoceanic shipment. When the analysis of variance revealed a significant ($P < 0{\cdot}05$) effect, Duncan's (1955) Multiple Range test was employed for mean separation. Frequency distributions were calculated for all colour score data.

Two separate ANOVAs were performed. The first ANOVA included all samples analysed at each of the three geographical locations. Some of the samples were not evaluated at all of the locations and thus the second ANOVA included only those products repeatedly sampled at each of the locations. By comparing these analyses the effects of sample thawing (in Florida) could be determined.

RESULTS AND DISCUSSION

Microbiological enumerations of fresh beef tongues (Table 3) and livers (Table 4) prior to and following interstate and transoceanic shipment showed similar patterns

TABLE 3
AEROBIC PLATE COUNTS FOR BEEF TONGUES PRIOR TO AND FOLLOWING TRANSCONTINENTAL AND TRANSOCEANIC SHIPMENT

Aerobic plate count	*Statistical comparisons*	*Guymon, Oklahoma* (*count/cm²*(n))	*Location Jacksonville, Florida* (*count/cm²*(n))	*Zeist, The Netherlands* (*count/cm²*(n))
35 °C APC[a]	1	ND[b]	2·56[c] (14)	1·52[d] (34)
	2	ND	2·56[c] (14)	1·50[d] (14)
20 °C APC	1	3·10[c] (46)	2·82[d] (14)	2·67[d] (34)
	2	3·01[c] (14)	2·82[c,d] (14)	2·63[d] (14)
7 °C APC	1	ND	2·30[c] (12)	2·10[c] (34)
	2	ND	2·30[c] (12)	2·47[c] (14)

[a] APC = Aerobic plate count ($\log_{10}$).
[b] ND = Not determined due to limited facilities.
[c,d] Means on the same line bearing the same superscripts are not significantly different ($P > 0{\cdot}05$).
1 = Statistical comparison of entire population of tongues sampled at Guymon versus entire population of tongues sampled at Jacksonville versus entire population of tongues sampled at Rotterdam.
2 = Statistical comparison of only those tongues repeatedly sampled at each of the three geographical locations.

with regard to change in bacterial numbers. APCs at 35 °C on beef tongues showed a significant ($P < 0{\cdot}05$) decrease in number following overseas shipment. As can be noted from the two statistical comparisons, the thawing of beef tongues in Jacksonville did not appear to have any effect on the final bacterial count for the 35 °C APC in Zeist. The first statistical comparison for the 20 °C APC, which includes both tongues that were thawed and those that remained frozen at

TABLE 4
AEROBIC PLATE COUNTS FOR BEEF LIVERS PRIOR TO AND FOLLOWING TRANSCONTINENTAL AND TRANSOCEANIC SHIPMENT

Aerobic plate count	*Statistical comparisons*	*Guymon, Oklahoma* (*count/cm²*(n))	*Location Jacksonville, Florida* (*count/cm²*(n))	*Rotterdam The Netherlands* (*count/cm²*(n))
35 °C APC[a]	1	ND[b]	3·44[c] (15)	2·71[d] (26)
	2	ND	3·67[c] (11)	2·85[d] (11)
20 °C APC	1	3·15[c] (32)	3·15[c] (16)	2·88[c] (26)
	2	3·04[c] (12)	3·31[c] (12)	3·01[c] (11)
7 °C APC	1	ND	2·43[c] (16)	1·98[c] (26)
	2	ND	2·72[c] (12)	2·10[d] (11)

[a] APC = Aerobic plate count ($\log_{10}$).
[b] ND = Not determined due to limited facilities.
[c,d] Means on the same line bearing the same superscripts are not significantly different ($P > 0{\cdot}05$).
1 = Statistical comparison of entire population of livers sampled at Guymon versus entire population of livers sampled at Jacksonville versus entire population of livers sampled at Rotterdam.
2 = Statistical comparison of only those livers repeatedly sampled at each of the three geographical locations.

Jacksonville, revealed a significant ($P < 0{\cdot}05$) decrease following interstate transport. There was no significant ($P < 0{\cdot}05$) decrease evident after overseas shipment. The second statistical comparison for the 20 °C APC indicated similar results as the first comparison with the exception that the differences were not significant ($P > 0{\cdot}05$) following interstate transport. Although the two comparisons disagree in this regard, the difference appears to be of little practical significance. The large sample number in the first comparison probably had an effect on making the difference significant ($P < 0{\cdot}05$). The 7 °C APC revealed no significant decrease in bacterial numbers following overseas shipment. The two statistical comparisons were similar and, as with the 35 °C APC, the thawing of the beef tongues in Jacksonville did not appear to have any effect on the final bacterial count in Zeist.

There was a significant ($P < 0{\cdot}05$) decrease in the 35 °C APC for beef livers following storage and overseas shipment. The two statistical comparisons were in agreement, thereby supporting the conclusion that thawing the livers in Jacksonville did not appear to affect the 35 °C aerobic counts obtained in Zeist. There were no significant ($P > 0{\cdot}05$) differences noted in 20 °C APC for livers following either interstate transport or overseas shipment. Again, both statistical comparisons were in agreement. Statistical comparison 1 for 7 °C APC showed no significant ($P < 0{\cdot}05$) differences in bacterial counts following overseas shipment. Statistical comparison 2 revealed a significant ($P < 0{\cdot}05$) decrease in 7 °C APC for beef livers following overseas shipment. Although the comparisons were statistically different, as they were with the 20 °C APC for beef tongues, this difference is probably of little practical significance. Coliform and total *Enterobacteriaceae* counts on tongues and livers sampled in both Florida and The Netherlands were all less than ten organisms per square centimetre. Previously, USA pork livers shipped to The Netherlands were shown to have *Enterobacteriaceae* in 90% of the isolates (de Kruijf, 1979).

Freezing and frozen storage may result in a reduction of the number of viable microorganisms of a food, depending on microflora, freezing and frozen storage conditions and enumeration methods (Speck & Ray, 1977). A considerable reduction was found with the 35 °C APC in our study. Our results indicate that microorganisms which grow at 35 °C are more susceptible to frozen storage than microorganisms which grow at 7 °C and 20 °C. While the reduction in bacterial numbers between Florida and The Netherlands was significant ($P < 0{\cdot}05$), this reduction was less than one $\log_{10}$ cycle and is probably of little practical significance. It would appear that frozen beef livers and tongues can be shipped for 72 h at above freezing temperatures (4·5 °C) without any appreciable change in aerobic bacterial numbers. Work by de Kruijf (1979) indicated that temperatures below 4 °C are required for good bacteriological quality with pork livers. He further concluded that pork livers from the USA that were rejected by The Netherlands authorities had higher bacterial counts than pork livers from the USA that were accepted by The Netherlands authorities.

Shelf-life evaluations

The distribution of colour scores for beef tongues initially (Guymon) as well as after transcontinental shipment (Jacksonville) is given in Fig. 1. Light brown (No. 5), light reddish brown (No. 6) and vivid red (No. 16) were present initially on the fresh tongues; however, after interstate transport, light brown was virtually non-existent while light reddish brown (No. 6) did not occur at all. Moderate reddish brown (No. 14) did not occur at all initially, but accounted for over 30% of the observed colours following interstate transport. Following transoceanic shipment

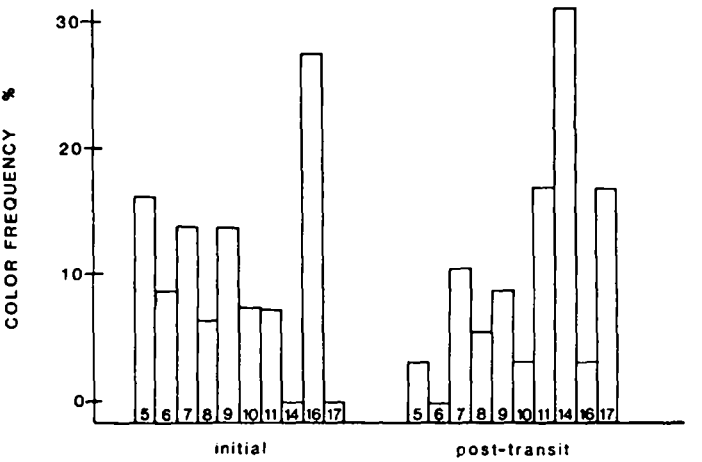

Fig. 1. Distribution of colour scores within each handling and storage condition for beef tongues prior to and following interstate transport. 5 = Light brown; 6 = Light reddish brown; 7 = Moderate reddish brown; 8 = Greyish red; 9 = Moderate reddish brown; 10 = Moderate reddish brown; 11 = Moderate reddish brown; 14 = Moderate reddish brown; 16 = Vivid red; 17 = Dark purplish red.

of the beef tongues to The Netherlands, moderate reddish brown (No. 14) was the most prevalent colour in 98% of the scores. It should be pointed out that the evaluators making up the panel in Zeist were not the same as those who evaluated the product initially and following transcontinental shipment. The evaluators in Zeist assigned only one colour score per sample; the most predominant colour.

The moderate reddish brown (No. 14) colour present on beef tongues after interstate and transoceanic shipment was probably due to the metmyoglobin pigment. Prolonged exposure to oxygen during transport could account for its presence. The vivid red (No. 16) colour is due to the formation of oxymyoglobin. This colour, which occurs frequently on fresh tongues, was oxidised to metmyoglobin during transit due to prolonged exposure to oxygen.

Off-odour scores for beef tongues prior to and following interstate and transoceanic shipment are presented in Table 5. Essentially no off-odour was detected initially or following interstate and overseas shipment of beef tongues.

TABLE 5
OFF-ODOUR SCORES FOR BEEF TONGUES AND LIVERS PRIOR TO AND FOLLOWING TRANSCONTINENTAL AND TRANSOCEANIC SHIPMENT

Location, Product	(n)	*Off-odour score*[a]
Shipment Origin, Guymon, Oklahoma		
Beef tongues	(60)	4·00
Beef livers	(42)	3·97
Jacksonville, Florida		
Beef tongues	(14)	3·96
Beef livers	(16)	3·96
Final Destination, Zeist, The Netherlands		
Beef tongues	(60)	4·00
Beef livers	(42)	4·00

[a] Scoring system identified in Table 2. Differences between locations for both products were non-significant ($P > 0{\cdot}05$).

Frozen storage appears capable of maintaining good odour quality for beef tongues for time periods similar to those of this study.

The distribution of colour scores for beef livers following interstate transport is presented in Fig. 2. Moderate reddish brown (No. 11) and dark red (No. 12) occurred more frequently on fresh livers than they did on the post-transit livers. Moderate reddish brown (No. 14), which was the most predominant colour initially,

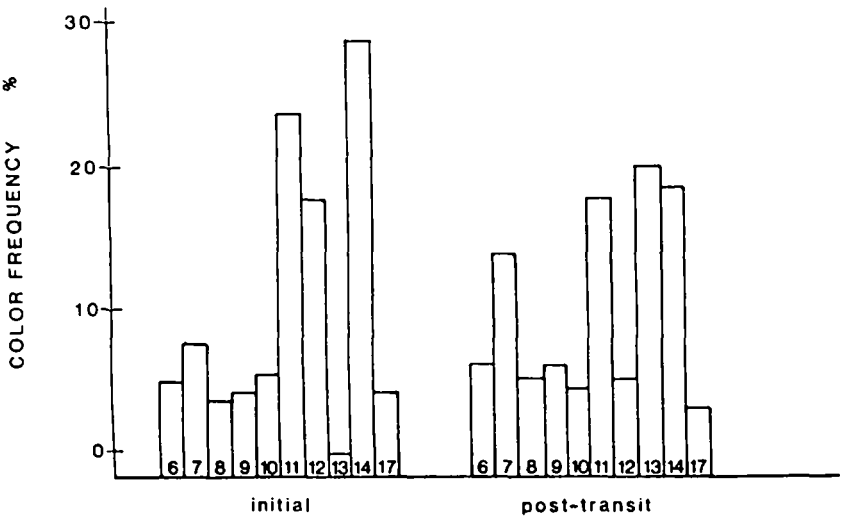

Fig. 2. Distribution of colour scores within each handling and storage condition for beef livers prior to and following interstate transport. 6 = Light reddish brown; 7 = Moderate reddish brown; 8 = Greyish red; 9 = Moderate reddish brown; 10 = Moderate reddish brown; 11 = Moderate reddish brown; 12 = Dark red; 13 = Blackish purple; 14 = Moderate reddish brown; 17 = Dark purplish red.

decreased in frequency after transit. The most dramatic change in the colour of beef livers was the frequency of the blackish purple (No. 13) colour. Blackish purple (No. 13) did not occur initially but accounted for approximately 20% of the assigned colours following interstate transport. Surface dehydration is the probable cause for the presence of this colour. Apart from this single substantial change, interstate shipment of beef livers does not appear to greatly affect the surface colours. The storage temperature, at which this product was kept during transport, appears to be able to maintain the fresh beef liver colours.

The predominant colours following transoceanic shipment of beef livers were moderate reddish brown (No. 11) which was the predominant colour for 50% of the livers, and dark red (No. 12) which was the predominant colour for 21% of the livers. Again, in Zeist, only one score was assigned per sample, that being the single most predominant colour.

Off-odour scores for beef livers prior to and following interstate and transoceanic shipment are presented in Table 5. Results for beef livers were similar to those of beef tongues. Virtually no off-odour was detected initially or following interstate and overseas shipment. Uniformity of colour for beef livers did not change significantly following transoceanic shipment. Beef livers had an average score of 4·1 (slightly uneven) prior to shipment and 4·6 (slightly uneven to slightly uniform) following overseas transit.

Weight loss of beef tongues and livers following interstate and overseas transport

Fresh beef tongues and livers were weighed initially at Guymon, and following shipment to Zeist. Beef tongues had a mean weight of 1·78 kg prior to transport and 1·76 kg after transit. Beef livers weighed 6·37 kg initially and 6·38 kg after overseas shipment. Overseas shipment of frozen beef tongues and livers does not appear to have any significant effect on the final weights of these products. Packaging of tongues and livers, similar to that of this study (commercial conditions) does not appear conducive to dehydration during frozen storage and shipment.

CONCLUSIONS

Generally, bacterial numbers on variety meats decreased during the transportation and storage phases of this study. The temperature abuse imposed on the variety meats during interstate transport and microbial sampling in Florida was not conducive to bacterial proliferation. Thus, in instances where bacterial growth has occurred on variety meats arriving in Europe, the abuse conditions must be more severe than those of this study. Shelf-life quality (odour, colour) of beef tongues and livers following transcontinental and oceanic shipment did not decrease significantly. The colour differences noted between the fresh and frozen product were probably due to the freezing, rather than the shipping and storage, conditions.

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